

aggressively and identified unaffected persons can be spared annual invasive and expensive clinical screening.

The prospect of a molecular diagnostic test for familial breast cancer was thought to be imminent with the cloning of the *BRCA1* gene. Unfortunately, the evidence thus far indicates that the quest for a comprehensive yet economically feasible DNA test for at-risk persons is formidable. The first difficulty stems from the indication that mutations in *BRCA1*, most likely a tumor-repressor gene, are extremely heterogeneous, with most of those identified so far unique to each family. With no predominant mutation(s) to screen for, each of the 22 exons in the *BRCA1* gene must be sequenced and analyzed. This will be enormously expensive and time-consuming. Second, *BRCA1*, although dominantly inherited, is not fully penetrant. Each of the five affected families initially studied had at least one female member older than 80 years positive for a *BRCA1* mutation but with no detectable breast cancer. Thus, mutations in *BRCA1* do not indicate a 100% probability of breast cancer developing, and their presence may not necessitate prophylactic mastectomy. Third, familial breast cancer is estimated to account for only 10% of all cases of breast cancer. The *BRCA1* gene is predicted to occur in 45% of cases of familial breast cancer; a second gene, *BRCA2*, apparently occurs in a similar proportion. A normal, nonmutated *BRCA1* gene does not indicate freedom from disease; a woman may get breast cancer related to *BRCA2*, or some other gene, and she is still susceptible to sporadic breast cancer. For all these reasons, molecular diagnostic screening for breast cancer predisposition presents extraordinary technical challenges; the results obtained raise clinical and ethical dilemmas.

Molecular diagnostic testing is now in its infancy with regard to two colorectal cancer syndromes. The first, familial adenomatous polyposis, is an autosomal, dominantly inherited, colorectal cancer syndrome with the hallmark of multiple adenomatous polyps (>100) that progress into cancer. The gene, adenomatous polyposis coli (*APC*), has been mapped to chromosome 5q21. Most kindreds have unique mutations in the *APC* gene, but two mutations occur in about 30% of cases. Molecular screening at this time involves linkage studies by DNA polymorphisms and direct sequencing of the gene. The second colorectal cancer syndrome, hereditary nonpolyposis colorectal cancer, is an autosomal dominant disorder that accounts for 4% to 6% of all colorectal cancers. The primary responsible genes (mismatch repair genes) are located on chromosome 2 (*HMSH2*) and chromosome 3 (*HMLH1*), but there are apparently others as well. Currently, linkage studies are the only feasible approach in most families, although functional protein-based assays are being developed.

Each week the literature is peppered with reports about the *p53* tumor-repressor gene. It is responsible for programmed cell death and cell-cycle arrest, and it has been linked to cancers of the bladder, breasts, lungs, colon, brain, and other tissues. Although germline *p53* mutations have been linked to a small number of familial

cancers (such as the Li-Fraumeni syndrome), somatic mutations can be found in sporadic tumors where they may aid in disease prognosis. For example, it was shown recently that tumors lacking wild-type *p53* were resistant to anticancer agents. Currently both DNA-based and immunohistochemical techniques can be used to detect mutated *p53*.

Diagnostic molecular oncology is evolving at a rapid pace, driven by the explosion of knowledge about the molecular biology of cancer and the frenzied cloning of disease genes under the impetus of the human genome project. But as new DNA tests emerge, diagnostic and ethical guidelines need to be developed and adhered to, despite the economic incentives and public appetite for this rapidly expanding field of pathology.

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Predisposition to Thrombosis by a Factor V Mutation Causing Hereditary Resistance to Activated Protein C

THROMBOEMBOLIC DISEASES resulting from the inappropriate activation of the blood coagulation cascade represent a major cause of morbidity and mortality worldwide, affecting about 1 in 1,000 people per year. The most common of these syndromes are deep venous thrombosis and its direct sequela, pulmonary embolism. Although specific risk factors for venous thrombosis have been well characterized (prolonged immobility, estrogens, major operations, trauma), genetic factors play a pathogenic role in at least a third of cases. Inherited mutations within the genes for the anticoagulant proteins antithrombin III, protein C, and protein S together account for only 5% to 10% of these thrombosis-prone families. A much larger number of these families have recently been found to have hereditary resistance to activated protein C, resulting from a highly prevalent missense mutation within the gene for coagulation factor V. The laboratory detection of this resistance to activated protein C is of clinical utility, as affected persons represent an important proportion of those with sporadic or recurrent thrombosis who may then benefit from prophylactic anticoagulant therapy.

Because activated protein C normally inhibits fibrin formation by cleaving and inactivating coagulation factors Va and VIIIa, the addition of activated protein C to normal plasma substantially prolongs the clotting time. In

contrast, the addition of activated protein C to the plasma from patients with hereditary resistance to activated protein C does not adequately prolong the clotting time. The functional laboratory test for this disorder is therefore an "activated protein C resistance ratio," a ratio between two activated partial thromboplastin times (aPTTs), one with the addition of activated protein C and one without. Activated protein C resistance ratio testing on families with histories of thrombophilia has shown that this phenotype is inherited as an autosomal dominant trait.

The genetic basis for hereditary resistance to activated protein C has recently been defined as a single point mutation in coagulation factor V at the site where activated protein C normally cleaves and inactivates the factor Va procoagulant. One or both copies of the factor V gene from patients with resistance to activated protein C thus carry a single G to A missense mutation converting arginine 506 to glutamine. After activation, this mutated factor Va cannot be efficiently cleaved by activated protein C; its procoagulant activity therefore persists, and the risk of thrombosis subsequently increases. The increased thrombotic risk associated with the heterozygous factor V mutation appears to be about five to ten times that of the normal population. The risk for patients carrying a homozygous factor V mutation is substantially greater.

The codon 506 factor V mutation is by far the most common genetic risk factor for venous thrombosis. About 20% of patients with a first episode of deep venous thrombosis and 60% of patients with recurrent or pregnancy-associated thromboembolism have functional resistance to activated protein C; the vast majority of these defects result from the codon 506 factor V mutation. This mutation is also prevalent in normal persons with heterozygote frequencies of 2% to 8% in different populations that have been screened. Of 197 randomly selected Red Cross blood donors in Portland, Oregon, for example, 15 (7.6%) were heterozygous for the codon 506 factor V mutation. The absence of disease in most of those carrying this mutation implies that, for most people, the formation of symptomatic thrombosis requires additional factors over and above this single defect. A two-hit hypothesis for thrombosis might explain some of these observations; patients with heterozygous resistance to activated protein C may then suffer thrombotic episodes only when additional thrombotic risks (genetic, physical, or biochemical) exist simultaneously. Examples of conditions likely to be synergistic with this disorder for symptomatic thrombosis include a second copy of the mutated factor V allele (that is, homozygosity), protein C or S deficiency, antithrombin III deficiency, the presence of a lupus anticoagulant, and homocystinemia. In support of this two-hit hypothesis, it has recently been shown that of heterozygous persons with activated protein C resistance, more than 70% with a simultaneous factor V mutation suffered a thrombotic event compared with only 30% of those without this second deficit.

Laboratory tests for the diagnosis of activated protein C resistance include the functional activated protein C resistance ratio test and the direct DNA-based detection of

the factor V codon 506 mutation. The presence of this mutation can be directly determined by the isolation of DNA from peripheral blood leukocytes, PCR amplification with primers flanking the factor V mutation site, and digestion of the PCR product with the restriction enzyme *MnII*. As the mutation destroys one of the *MnII* sites within this amplicon, the mutant and wild-type alleles can be easily distinguished on a size-separating gel. In our laboratory, the correlation between the functional and DNA-based tests has been excellent. Anticoagulated patients cannot, unfortunately, be evaluated by the activated protein C resistance ratio test (a modified aPTT); the DNA-based test is then the only diagnostic alternative for this group. Additional clinical indications for the DNA-based (rather than the functional) test for hereditary resistance to activated protein C include patients with lupus anticoagulants, those with borderline activated protein C resistance ratios, the evaluation of homozygotes versus heterozygotes, and the confirmation of genotype in affected persons and their family members.

The accurate laboratory identification of hereditary resistance to activated protein C is of real clinical relevance because some, but not all, of these patients may benefit from long-term anticoagulant therapy. In particular, those heterozygous patients with several previous thrombotic episodes, those with a known genetic "second hit" (including homozygotes), and some with a single "spontaneous" episode of deep venous thrombosis (no known environmental risk) should probably be treated with long-term anticoagulants. In contrast, most heterozygous persons with either no history of thrombosis or with a single, previous episode of deep venous thrombosis induced environmentally (surgery, trauma, or prolonged immobility) should not always be prophylactically treated. Instead, these patients should be thoroughly advised of the signs and symptoms of deep venous thrombosis or pulmonary embolism, encouraged to seek early medical attention for their suspected occurrence, and given optimal antithrombotic prophylaxis for surgical therapy, pregnancy, and prolonged immobility. Until definitive clinical studies on these patient groups are published, these therapeutic recommendations are based on the fact that in the great majority of persons heterozygous for activated protein C resistance, thrombosis does not develop until additional "secondary" events occur.

Because this highly prevalent genetic mutation was identified only recently, the full spectrum of its clinical manifestations remains unknown. Does the highly prevalent factor V mutation, for example, contribute to the arterial thrombotic episodes that cause myocardial infarction and cerebrovascular disease? The absence of an increased frequency of heterozygotes in a population of elderly stroke patients suggests that this mutation may not predispose to common arterial thrombotic events. Alternatively, should laboratory screening for hereditary resistance to activated protein C be instituted in thrombosis-prone populations (pregnant women, patients with atrial fibrillation, and before major orthopedic operations) to identify those who might benefit from prophylactic an-

ticoagulants? The answers to these and other pertinent clinical questions may allow us to direct antithrombotic therapy at those most likely to have thrombosis and, perhaps more important, to avoid it in others.

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Microalbuminuria

THE DEVELOPMENT of clinically serious nephropathy is a common complication of diabetes mellitus. At least 30% of new patients with end-stage renal disease have diabetic nephropathy. Among these cases, half have insulin-dependent diabetes mellitus (IDDM) and half have non-insulin-dependent diabetes (NIDDM).

The urinary albumin excretion in normal young subjects is less than 10 µg per minute (about 15 mg per day), but it increases slowly with age. Overt or clinically detectable proteinuria is commonly defined as a urinary albumin excretion rate greater than 200 µg per minute (about 300 mg per day). Thus, there exists an intermediate range of 20 to 200 µg per minute (30 to 300 mg per day) that is definitely not normal and is associated with major clinical findings. This persistent increase in subclinical urinary albumin excretion has been called "microalbuminuria." Hence, the term does not imply excretion of a variant of albumin but of smaller quantities than those detectable by traditional dipstick methods.

Microalbuminuria is strongly associated with the eventual development of nephropathy in 80% of patients with IDDM and 25% of patients with NIDDM. It is also associated with increased morbidity and mortality. Those IDDM patients with microalbuminuria who were treated for hypertension had substantial decreases in the urinary albumin excretion rate and a pronounced decrease in the rate of decline of renal function. Thus, microalbuminuria is an early marker for the development of diabetic nephropathy and possibly for increased mortality as well. The determination of this condition in patients who are "dipstick-negative" could be clinically important. It has been recommended that microalbuminuria be determined annually in patients with IDDM of more than five years' duration and in all patients with NIDDM. The detection of notable microalbuminuria in these patients may warrant altered treatment, even if other variables (such as blood glucose or glycated hemoglobin levels) are not substantially abnormal.

Although the primary indication for monitoring microalbuminuria is to evaluate the renal status in diabetes

mellitus, recent studies suggest that such measurement may also be helpful in patients with preeclampsia, drug nephrotoxicity, and severe hypertension.

Traditional urine dipstick methods are not sufficiently sensitive to detect mild to even moderate microalbuminuria. More than a quarter of patients with IDDM and a third of those with NIDDM who were negative for albumin by dipstick were found to have clinically substantial microalbuminuria.

A variety of sensitive quantitative methods are available for monitoring microalbuminuria. These include radioimmunoassay, enzyme-linked immunosorbent assay, and nephelometric immunoassays. The clinical chemistry laboratory at our medical center (University of California, San Diego) uses a rate nephelometric assay. The method detects as little as 2 µg per ml of albumin. The preferred specimen is a 24-hour urine collection because this permits the rate of albumin excretion over a protracted period of time to be calculated. Because collecting a 24-hour specimen is not always feasible, timed, short-term collections may be used. Randomly collected specimens should be avoided because the rate of excretion cannot be calculated. If such specimens must be used, the creatinine concentration should also be measured and the ratio of albumin to creatinine determined. The normal urine albumin:creatinine ratio is less than 0.01. Patients with microalbuminuria generally have values greater than 0.02. In patients with IDDM, there is a strong association between the ratio determined in a 4-hour collection and the 24-hour-urinary albumin excretion rate, with a high positive predictive value. The test costs about \$65.

In addition to the importance of timed specimens, urine specimens should not be collected during periods of excessive exercise or prolonged standing. Specimens should not be collected from patients with hematuria or urinary tract infection. Glass containers should not be used because albumin adheres to glass.

Even when these guidelines are observed, day-to-day urinary albumin excretion rates vary considerably. The intraperson coefficient of variation on a daily basis may be as high as 45%. Therefore, it has been suggested that patients have at least three timed urine collections over a six-month period. A patient would be considered to have microalbuminuria if the urinary albumin excretion were increased in two of the three specimens.

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